9. M. K. Jenkins, J. D. Ashwell, R. H. Schwartz, *J. Immunol.* **140**, 3324 (1988).
10. A. Vitiello, W. R. Heath, L. A. Sherman, *ibid.* **143**, 1512 (1989).
11. M. K. Jenkins, D. M. Pardoll, J. Mizuguchi, T. M. Chused, R. H. Schwartz, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5409 (1987).
12. J. D. Ashwell, R. E. Cunningham, P. D. Noguchi, D. Hernandez, *J. Exp. Med.* **165**, 173 (1987).
13. R. H. Schwartz, D. L. Mueller, M. K. Jenkins, H. Quill, *Cold Spring Harbor Symp. Quant. Biol.* **54**, 605 (1989).
14. After exposure to irradiated APCs plus T-OVA, GX1 cells showed enhanced cytotoxicity on EL4 targets in the presence of T-OVA [40% at an effector: target ratio of 3:1 in the presence of T-OVA (300 μg/ml) after stimulation, compared with 23% before]. These same activated cells proliferated after exposure to T-OVA and irradiated APCs (33,000 cpm compared with 47,000 cpm for cells not restimulated before assay, under the conditions described in Table 2).
15. F. Ramsdell, T. Lantz, B. J. Fowlkes, *Science* **246**, 1038 (1989); J. L. Roberts, S. O. Sharrow, A. Singer, *J. Exp. Med.* **171**, 935 (1990).
16. Recombinant IL-1 α (10 unit/ml) did not induce proliferation of GX1 cells that were stimulated with an optimal concentration of T-OVA plus paraformaldehyde-fixed B6 spleen cells.
17. T. Azuma *et al.*, *J. Immunol.* **143**, 1 (1989).
18. A. S. Rosenberg, T. Mizuochi, A. Singer, *J. Exp. Med.* **168**, 33 (1988).
19. A. Weiss *et al.*, *Annu. Rev. Immunol.* **4**, 593 (1986).
20. E. D. Hsi *et al.*, *J. Biol. Chem.* **264**, 10836 (1988).
21. G. R. Crabtree, *Science* **243**, 355 (1989).
22. G. LeGros, S. Gillis, J. D. Watson, *J. Immunol.* **135**, 4009 (1985).
23. P. E. Baker, S. Gillis, K. A. Smith, *J. Exp. Med.* **149**, 273 (1979).
24. O. Leo, M. Foo, D. H. Sachs, L. E. Samelson, J. A. Bluestone, *Proc Natl. Acad. Sci. U.S.A.* **84**, 1374 (1987).
25. We thank B. J. Fowlkes, W. E. Paul, R. H. Schwartz, E. M. Shevach, and A. Singer for discussion and comments. G.R.O. is supported by the American Cancer Society.

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Regulation of Phospholipase C–γ1 by Profilin and Tyrosine Phosphorylation

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Epidermal growth factor and platelet-derived growth factor can stimulate the production of the second messenger inositol trisphosphate in responsive cells, but the biochemical pathway for these signaling events has been uncertain because the reactions have not been reconstituted with purified molecules in vitro. A reconstitution is described that requires not only the growth factor, its receptor with tyrosine kinase activity, and the soluble phospholipase C–γ1, but also the small soluble actin-binding protein profilin. Profilin binds to the substrate phosphatidylinositol 4,5-bisphosphate and inhibits its hydrolysis by unphosphorylated phospholipase C–γ1. Phosphorylation of phospholipase C–γ1 by the epidermal growth factor receptor tyrosine kinase overcomes the inhibitory effect of profilin and results in an effective activation of phospholipase C–γ1.

CELL GROWTH REQUIRES THE COORdinated regulation of multiple biochemical systems including those leading to DNA synthesis and the reorganization of the cytoskeleton. It is remarkable that the whole process can be initiated by the binding of an extracellular factor such as epidermal growth factor (EGF) or platelet derived-growth factor (PDGF) to a single class of membrane receptors. How these receptors are coupled to the biochemical processes they induce is not well understood. For example, it is not clear how binding of EGF to its receptor is coupled to the production of the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG).

The EGF receptor (EGFR) is a transmembrane protein with an EGF binding site outside the cell and a tyrosine kinase catalytic domain inside the plasma membrane (*1*). Upon binding EGF, the receptor dimerizes and phosphorylates tyrosine residues on itself and on several effector proteins likely to participate in signal transduction (*1, 2*). Some effector proteins also become physically associated with the receptor (*1, 2*). One such effector is phosphatidylinositide (PI)-specific phospholipase C–γ1 (PLC-γ1), a soluble enzyme in quiescent cells (*3, 4*). This enzyme catalyzes the hydrolysis phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce IP_3 and DAG (*5*). In some cells, EGF and PDGF stimulate the phosphorylation of PLC-γ1 on tyrosine, the binding of PLC-γ1 to the EGFR or to the PDGF receptor (PDGFR), and the production of IP_3 (*3*).

The biochemical connections among these events are not well understood. Most studies have shown that the catalytic activity of purified PLC-γ1 is not altered by tyrosine phosphorylation (*6*). However, in one study PLC-γ1 was activated when phosphorylated on tyrosine by the EGF receptor; in this case immunoprecipitated PLC-γ1 was used and the substrate consisted of PIP_2 in mixed micelles with two detergents (*7*).

The role of profilin, a cytoplasmic actin binding protein, in the PI signaling pathway has been equally mysterious. Profilin from human platelets binds four to five molecules of PIP_2 or phosphatidylinositol 4-monophosphate with relatively high affinity but does not bind other lipids (*8, 9*). Binding of

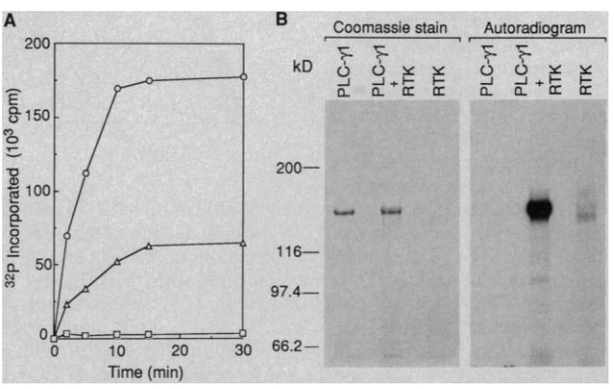

Fig. 1. Phosphorylation of PLC-γ1 by the EGF receptor. (**A**) Time course of phosphorylation by EGFR of PLC-γ1 (○), or of itself in the absence of PLC-γ1 (△). Control samples contained PLC-γ1 but no EGFR (□). The phosphorylation reaction was initiated by addition of ATP. At indicated time points, a 5-μl portion of the reaction mixture was removed and analyzed for phosphoprotein content (*6*). (**B**) Analysis by SDS–polyacrylamide gel electrophoresis (6% gels) and autoradiography of the protein and phosphoprotein content of the reaction mixtures. The lanes contain 30 μl of each reaction mixture, collected 30 min after the initiation of the phosphorylation reaction. Molecular mass markers (in kilodaltons) are indicated. Phosphorylation was performed at 22°C in 60 μl of a solution containing 20 mM Hepes, pH 7.4, 25 mM $MgCl_2$, 4 mM $MnCl_2$, 0.1 mM Na_3VO_4, 100 ng of EGF, and 10 μM [γ-^{32}P]ATP (1.5 Ci/mmol), in the presence of 5 μg of PLC-γ1 and 20 ng of lectin-purified EGFR (*6, 19*). This experiment is representative of three experiments performed with EGFR and four experiments performed with truncated recombinant EGFR (20 ng) corresponding to the cytoplasmic tyrosine kinase domain (*13*).

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profilin to these lipids inhibits the interaction between profilin and actin (*10, 11*). In addition, binding of profilin to PIP_2 inhibits the hydrolysis of PIP_2 by soluble PLCs (*8, 9*). Given the high concentration of profilin in cells, it has not been clear how the PLCs gain access to their substrates.

We have now reconstituted EGF-regulated production of IP_3 with purified proteins and synthetic membranes. The previously unrecognized feature is that the binding of profilin to the substrate PIP_2 inhibits unphosphorylated but not phosphorylated PLC-γ1. Consequently, activation of the EGFR tyrosine kinase can increase the production of IP_3 by phosphorylating PLC-γ1. We hypothesize that the same mechanism may control the production of IP_3 and DAG in live cells.

The activated EGFR phosphorylated, on average, one tyrosine residue per PLC-γ1 molecule in 15 min (Fig. 1). This reaction required EGF, EGFR, and adenosine triphosphate (ATP) and reached a plateau after 15 min of incubation (Fig. 1) (*6, 12*). Profilin was not phosphorylated by the EGFR under these conditions.

In the absence of profilin, phosphorylation of PLC-γ1 had only a small effect on its activity when tested with the substrate PIP_2 in a phospholipid bilayer (Fig. 2A) (*6*). However, in the presence of profilin, the activity of PLC-γ1 was dependent on phosphorylation (Fig. 2). Inhibition of unphosphorylated PLC-γ1 depended on the concentration of profilin (Fig. 2B). If PIP_2 bound to profilin is not available as a substrate for unphosphorylated PLC-γ1, the dependence of the activity of unphosphorylated PLC-γ1 on the concentration of profilin can be predicted from the established affinity and stoichiometry of profilin binding to PIP_2 (*8, 9*) (Fig. 2). Phosphorylated PLC-γ1 was actually activated slightly (Fig. 2B) by physiological concentrations of profilin (~50 μM) (*11*). Experiments with a highly purified truncated form of the EGFR (*13*) consisting of only the receptor tyrosine kinase (RTK) cytoplasmic domain gave similar results. It phosphorylated the same sites on PLC-γ1 (*14*), and this phosphorylated enzyme was not inhibited by profilin (Fig. 2B).

It is not yet clear how tyrosine phosphorylation of PLC-γ1 might allow the enzyme to overcome the inhibitory effect of profilin bound to its substrate PIP_2, but the inability of profilin to inhibit IP_3 production by PLC-β (Fig. 3) provides some clues. PLC-β and PLC-γ1 have similar substrate specificities, but they have different subcellular distributions. Whereas most of the PLC-γ1 is in the cytoplasm of resting cells, ~80% of the PLC-β is associated with the particulate fractions (*14, 15*). Analysis of the amino acid sequence of PLC-β failed to demonstrate the presence of a membrane spanning domain. Post-translational modification of the enzyme is likely to be responsible for its cellular localization (*4*). It is possible that the association of PLC-β with membranes is responsible for the insensitivity of the enzyme to inhibition by profilin.

In cells, phosphorylation of PLC-γ1 by RTK is accompanied by a shift from the soluble fraction to an insoluble fraction containing membranes (*16*), and such an association of phosphorylated PLC-γ1 with membranes might relieve the inhibitory effects of profilin. One way that phosphorylated PLC-γ1 might associate with membranes is to bind to the EGF receptor (*1–3*). This is unlikely to account for our results since the receptor was present only in catalytic concentrations (Fig. 1), and the truncated RTK is unlikely to associate with membranes. Alternatively, phosphorylated PLC-γ1 might simply have a higher affinity for PIP_2 that has not been detected in previous assays with pure lipid substrates (*6*), or it might interact in some way with other phospholipids, with other PLC-γ1 molecules, or with profilin.

We propose that profilin may be part of the mechanism coupling the binding of EGF (and possibly PDGF) to its receptor to the production of IP_3. Prior to activation, profilin inhibits soluble PLC-γ1 by binding to its substrate PIP_2. Activation of the receptor by EGF results in the phosphorylation of PLC-γ1 which is then enzymatically active even in the presence of profilin.

The proposed signaling mechanism including profilin may explain some of the effects of EGF and PDGF, but other mechanisms must couple the binding of growth factors to their receptors with the full range of responses required for mitogenesis (*1, 2, 17*). For example, in NIH 3T3 cells transfected with PLC-γ1 (*18*), the basal rate of PI turnover was similar to that of control NIH 3T3 cells, suggesting that the overexpressed

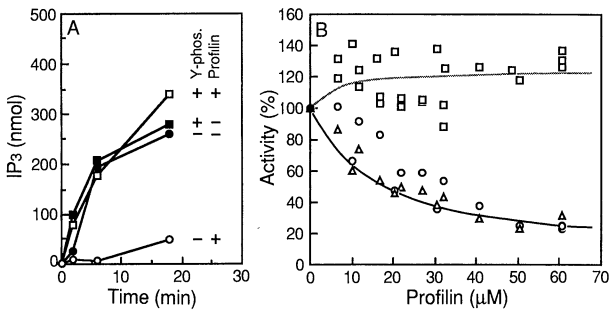

Fig. 2. Effect of profilin and tyrosine kinase phosphorylation of PLC-γ1 on the hydrolysis of PIP_2. Samples of PLC-γ1 were phosphorylated by incubation with EGF-activated EGFR (A) or RTK (B) for 15 min (Fig. 1) before phospholipase activity was measured. Unphosphorylated controls were obtained by omitting either ATP or the tyrosine kinase (EGFR or RTK) from the incubation. PLC-γ1 activity (*20*) was measured at 22°C in 100 μl of a solution containing 5 mM tris, pH 7.0, 75 mM KCl, 0.5 mM dithiothreitol, 0.4 mM EGTA, 0.2 mM $CaCl_2$, 0.1 mM NaN_3, a 1:20 dilution of the phosphorylation reaction (30 nM PLC-γ1, final concentration), and variable concentrations of profilin (*19*). (**A**) Time course of IP_3 production from large unilamellar vesicles (*21*) composed of 20 μM [^{3}H]PIP_2 (0.1 Ci/mol), 100 μM phosphatidylethanolamine (PE), and 50 μM phosphatidylserine (PS) without or with profilin (36 μM). The specific activity of unphosphorylated PLC-γ1 was 21.9 ± 4.1 nmol min^{-1} mg^{-1} (mean ± SD of four separate experiments, each performed in duplicate) in the absence of profilin; and 3.8 ± 2.0 nmol min^{-1} mg^{-1} in the presence of profilin, the specific activity of phosphorylated PLC-γ1 was 34.7 ± 5.3 nmol min^{-1} mg^{-1} in the absence of profilin; and 33.2 ± 8.0 nmol min^{-1} mg^{-1} in the presence of profilin. (**B**) Dependence of the production of IP_3 from large unilamellar vesicles of [25 μM [^{3}H]PIP_2 (0.1 Ci/mol), 100 μM PE, 50 μM PS, and 100 μM phosphatidylcholine (PC)] on the concentration of profilin during a 5-min incubation. PLC-γ1 was first incubated with RTK and ATP (□), ATP but no RTK (○), or RTK but no ATP (△). The two theoretical curves were based on the following assumptions: profilin binds to PIP_2 pentamers with a dissociation constant (K_d) of 3 μM (*8, 9*); PIP_2 bound to profilin is not available as a substrate for hydrolysis by unphosphorylated PLC-γ1; unphosphorylated PLC-γ1 has a Michaelis constant (K_m) of 1.25 μM for free PIP_2; the V_{max} for PIP_2 hydrolysis by phosphorylated PLC-γ1 is 25% higher for PIP_2 bound to profilin than for free PIP_2.

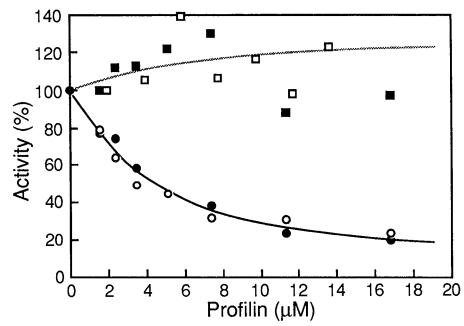

Fig. 3. Comparison of the effect of profilin on PLC-γ1 and PLC-β activity. Assays were conducted using PLC-β (□) or PLC-γ1 (○) (4 μg/ml) for 5 min at 22°C in buffer (5 mM tris, pH 7.0, 75 mM KCl, 25 μM $CaCl_2$, 0.5 mM dithiothreitol, 0.1 mM NaN_3). The substrate consisted of large unilamellar vesicles of 30 μM [^{3}H]PIP_2 (0.1 Ci/mol), 50 μM PC and 50 μM PE. Open and solid symbols represent two separate experiments. The specific activities in the absence of profilin were 46.5 nmol min^{-1} mg^{-1} for PLC-γ1 and 37.8 nmol min^{-1} mg^{-1} for PLC-β. The theoretical curves were calculated by assuming that profilin binds to PIP_2 pentamers with a K_d of 3 μM, that PLC-γ1 hydrolyzes only free PIP_2, and that PLC-β hydrolyzes PIP_2 bound to profilin 25% faster than free PIP_2.

PLC-γ1 was inhibited by a factor present in these cells (possibly profilin). In response to PDGF, tyrosine phosphorylation of overexpressed PLC-γ1 was associated with a higher rate of PI turnover than that observed in untransfected NIH 3T3 cells. However, neither the transient increase in concentration of cytoplasmic Ca^{2+} nor the rate of synthesis of DNA was affected. One interpretation is that activation of PLC-γ1 might control a pathway independent of that leading to the synthesis of DNA (*17, 18*), such as the pathway responsible for the reorganization of the cytoskeleton. Profilin released from membranes after hydrolysis of PIP_2 by activated PLC-γ1 could participate in such a response.

REFERENCES AND NOTES

1. A. Ullrich and J. Schlessinger, *Cell* **61**, 203 (1990); G. Carpenter and S. Cohen, *J. Biol. Chem.* **265**, 7709 (1990).
2. L. T. Williams, *Science* **243**, 1564 (1989); C.-H. Heldin and B. Westermark, *Cell Regul.* **1**, 555 (1990).
3. M. I. Wahl, T. O. Daniel, G. Carpenter, *Science* **241**, 968 (1988); B. Margolis *et al.*, *Cell* **57**, 1101 (1989); J. Meisenhelder, P.-G. Suh, S. G. Rhee, T. Hunter, *ibid.*, p. 1109; B. Margolis *et al.*, *Mol. Cell. Biol.* **10**, 435 (1990); D. K. Morrison, D. R. Kaplan, S. G. Rhee, L. T. Williams, *ibid.*, p. 2359.
4. S. G. Rhee, P.-G. Suh, S.-H. Ryu, S. Y. Lee, *Science* **244**, 546 (1989).
5. M. J. Berridge and R. F. Irvine, *Nature* **341**, 197 (1989); Y. Nishizuka, *ibid.* **334**, 661 (1988).
6. J. W. Kim *et al.*, *J. Biol. Chem.* **265**, 3940 (1990).
7. S. Nishibe, M. I. Wahl, N. K. Tonks, S. G. Rhee, G. Carpenter, *Science* **250**, 1253 (1990).
8. P. J. Goldschmidt-Clermont, L. M. Machesky, J. J. Baldassare, T. D. Pollard, *ibid.* **247**, 1575 (1990).
9. L. M. Machesky, P. J. Goldschmidt-Clermont, T. D. Pollard, *Cell Regul.* **1**, 937 (1990).
10. I. Lassing and U. Lindberg, *Nature* **314**, 472 (1985); *J. Cell Biochem.* **37**, 255 (1988).
11. P. J. Goldschmidt-Clermont, L. M. Machesky, S. K. Doberstein, T. D. Pollard, *J. Cell. Biol.*, in press.
12. The level of phosphorylation of PLC-γ1 plateaued (~1 phosphate per PLC-γ1 molecule) after 15 min and remained constant for the next 75 min (*6*). The experimental conditions were selected to optimize the phosphorylation of the tyrosine residue 783 of PLC-γ1, an important substrate for the EGFR and PDGFR (S. G. Rhee, unpublished data). However, other tyrosine residues of PLC-γ1 can also be phosphorylated by the EGFR or RTK in vitro (*6*), and it is likely that our phosphorylated PLC-γ1 preparations were heterogeneous and contained mainly the monophosphorylated PLC-γ1 but also some unphosphorylated and multiphosphorylated enzyme. The PIP_2 hydrolysis assays using the phosphorylated enzyme preparation were all performed within 15 to 35 min after the initiation of the phosphorylation reaction.
13. C. Y. C. Hsu *et al.*, *Cell Growth Differ.* **1**, 191 (1990).
14. S. G. Rhee, unpublished results.
15. K.-Y. Lee, S. H. Ryu, P.-G. Suh, W. C. Choi, S. G. Rhee, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5540 (1987); H. R. Carter, M. A. Wallace, J. N. Fain, *Biochim. Biophys. Acta* **1054**, 119 (1990).
16. G. Todderud, M. I. Wahl, S. G. Rhee, G. Carpenter, *Science* **249**, 296 (1990).
17. M. Whitman and L. Cantley, *Biochim. Biophys. Acta* **948**, 327 (1988); T. D. Hill *et al.*, *Science* **248**, 1660 (1990).
18. B. Margolis *et al.*, *Science* **248**, 607 (1990).
19. Profilin was purified from outdated human platelets [D. A. Kaiser, P. J. Goldschmidt-Clermont, B. A. Levine, T. D. Pollard, *Cell Motil. Cytoskeleton* **14**, 251 (1989)] by affinity chromatography on a poly-L-proline agarose column, elution with urea, and renaturation by dialysis against buffer (5 mM tris, pH 7.5, 75 mM KCl, 0.5 mM dithiothreitol, and 0.1 mM NaN_3). PLC-γ1 and PLC-β were purified from bovine brain and stored at −70°C [S. H. Ryu, K. S. Cho, K.-Y. Lee, P.-G. Suh, S. G. Rhee, *J. Biol. Chem.* **262**, 12511 (1987)]. EGFR from A-431 cells was prepared by affinity chromatography of cell extracts on wheat germ lectin Sepharose 6 MB (Pharmacia) (*6*).
20. Each PLC assay was performed in a final volume of 100 μl (*8, 9*). At the end of the incubation, the reaction was stopped by addition of 375 μl of an ice-cold solution of methanol and chloroform (2:1). Further lipid extraction was performed by addition of 125 μl of chloroform and 125 μl of 1 M HCl. The samples were mixed and centrifuged (1000*g* for 5 min at room temperature), and the top 200 μl of the aqueous phase was collected for scintillation counting.
21. All lipids were from Avanti Polar Lipids (Pelham), except PIP_2 (Calbiochem or Boerhinger) and phosphatidyl-[2-^{3}H]inositol 4,5-bisphosphate ([^{3}H]PIP_2) (Amersham). Large unilamellar vesicles made of PIP_2 mixed with other phospholipids were prepared by the extrusion technique (*8, 9*). The concentration of lipid in each mixture was measured by liquid scintillation counting of part of the sample after extrusion.

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Enhanced Motility in NIH 3T3 Fibroblasts That Overexpress Gelsolin

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Increasing the content of the actin-binding protein gelsolin in cultured mouse fibroblasts by up to 125 percent by gene transfection proportionally enhanced the rate at which the cells migrated through porous filters toward a gradient of serum and closed a wound made on a confluent monolayer of cells in a tissue culture dish. These results provide direct evidence that gelsolin, which promotes both actin assembly and disassembly in vitro, is an important element in fibroblast locomotion and demonstrate that the manipulation of intracellular machinery can increase cell motility.

EUKARYOTIC CELL LOCOMOTION depends on the assembly and disassembly of actin polymers (*1*), a process regulated by actin-binding proteins (*2*). One such protein implicated in the control of this actin assembly cycle is gelsolin, because it can affect either actin assembly or disassembly and because calcium and polyphosphoinositides, which are intracellular signals, control its interactions with monomeric actin and filamentous actin (F-actin) in vitro. In the presence of micromolar Ca^{2+}, gelsolin promotes the rapid disassembly of F-actin by severing actin filaments, after which it remains tightly bound to the ends of the fragmented filaments (*3*). Membrane polyphosphoinositides, phosphatidylinositol 4-phosphate (PIP) or phosphatidylinositol 4,5,-bis-phosphate (PIP_2), dissociate gelsolin bound to the ends of F-actin, thereby generating free filament ends that act as nuclei for rapid assembly of monomeric actin into filaments (*4*). Actin filaments with ends blocked by gelsolin are therefore potentially key intermediates for eliciting actin assembly at locations where perturbation of cell surface receptors causes appropriate changes in polyphosphoinositide concentrations, conformations, or both. The dissociation of gelsolin-actin complexes precedes net actin assembly in agonist-stimulated cells (*5*), and gelsolin molecules, bound to short actin filaments, have been seen in electron micrographs of cell membranes (*6*), providing indirect evidence that gelsolin-capped actin oligomers may serve as nuclei to promote actin polymerization at the cell's edge.

In a cell stimulated by chemotactic agents, actin disassembly and assembly increase, which accelerates reorganization of the cytoskeleton required for the mechanics of cell movement. If the reversible association of gelsolin with actin regulated by calcium, polyphosphoinositides, and possibly other signals is important for the actin turnover between assembled and disassembled states, then it follows that the gelsolin concentration could be rate-limiting for this cycle. Furthermore, if gelsolin couples the cycle to locomotion through signals generated at the membrane, cell translocation could be quantitatively related to cellular gelsolin content. The observations we report here support this hypothesis.

To examine gelsolin's role in cell motility directly, we permanently transfected NIH 3T3 fibroblasts with the cDNA for human cytoplasmic gelsolin (*7*) in the β-actin promoter-driven expression vector LK444 (*8*), which confers resistance to G418 (*9*), and is designated LKCG. Selection for G418 resistance yielded a mixed population of cells

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